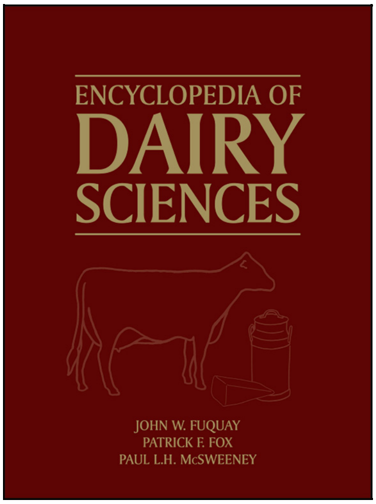

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Starter Cultures

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Introduction

Starter cultures were unknown until 1878, when Lister isolated pure cultures of the lactic acid bacteria responsible for milk acidification. In the 1880s, improvements to standardization and ripening of butter were made by introducing pure starter cultures (Storch in Denmark, Conn in the United States, and Weigmann in Germany). During the period 1910–20, Orla-Jensen, von Freudenreich, Sherman, Hammer, and other dairy microbiologists prepared pure cultures for fermented milks. Commercial production and use of starter cultures grew rapidly and was widespread at the beginning of the twentieth century.

Dairy starters are the 'heart' of fermented milk products, the most crucial component in the manufacture of high-quality fermented milks. The cultures are harmless food-grade microorganisms, such as active bacteria, that are intentionally grown in milk or whey or other formulated media to impart desirable and predictable flavor and texture to fermented milk products.

The microorganisms employed in milk fermentation are single-strain or multiple-strain cultures of lactic acid bacteria, producing different types of fermented milk products. Usually, one or two strains dominate the milk environment. Individual strains can be selected in advance for their resistance to both bacteriophages (phage) and antibiotics. Mixed starter cultures are used to ensure that fermentation will continue after a bacteriophage attack. Bacteriophages are highly strain specific, and if the dominant strain in a mixed-strain starter culture succumbs to an attack, the phage-resistant mutant or the next dominant culture maintains a satisfactory rate of lactic acid production.

Performance indicators of starter cultures include (1) adaptation to various manufacturing conditions, (2) rapid acid production in the vat, (3) minimal acid production during distribution and storage, (4) maintenance of viability during the shelf life of fermented milk, and (5) typical flavor, body, and texture formation.

Strains are selected for the rate of growth and lactic acid production, aroma and/or carbon dioxide production, resistance to phage, ability to produce viscous or ropy fermented milk, ability to maintain desirable ratios of component organisms, and viability during preparation of starter culture, preservation steps, storage, and distribution.

Starter cultures should contain the highest possible number of viable microorganisms, be highly active under production conditions, and be free from contaminants. The fermentation process of any cultured dairy product relies entirely on the purity and activity of the starter culture. In addition, milk or growth medium should not contain inhibitory agents, such as antibiotics and bacteriophage.

Types of Starter Cultures

Diverse lactic starter cultures are used in the manufacture of commercial fermented milk products in the world (**Table 1**). They can be classified into mesophilic cultures, which grow best at 25–30 °C, and thermophilic cultures, which grow at higher temperatures (37–45 °C).

Mesophilic Starter Cultures

Mesophilic cultures are widely used in the fermented milk industry (**Table 2**) in the manufacture of products such as 'filmjölk' and 'lactofil' (in Sweden) and 'ymer' (in Denmark). Mesophilic starters will almost certainly contain *Lactococcus lactis* subsp. *cremoris*, but rarely will this species be used alone. Buttermilk, 'långfil', and 'viili', which are popular in Norway, Sweden, and Finland, combine *Lc. lactis* subsp. *cremoris* with *Leuconostoc* species. Other fermented milk products made with mesophilic starters include sour cream, cultured buttermilk, and kefir (*see* **Lactic Acid Bacteria**: *Lactococcus lactis*; *Leuconostoc* spp.).

Thermophilic Starter Cultures

Thermophilic starter cultures (**Table 3**) are used for the manufacture of yogurt, Bulgarian buttermilk, and the whole range of products made with intestinal bacteria, primarily lactobacilli and bifidobacteria. Thermophilic cultures are classified into two main types: artisanal

Table 1 Primary starter cultures used for milk fermentations

Mesophilic	*Thermophilic*
Lactococcus lactis subsp. *lactis*	*Lactobacillus delbrueckii* subsp. *bulgaricus*
Lc. lactis subsp. *cremoris*	*Streptococcus thermophilus*
Lc. lactis subsp. *lactis* biovar *diacetylactis*	*Lb. helveticus*
Lactobacillus kefir	*Lb. acidophilus*
Lb. casei	*Lb. paracasei* subsp. *paracasei*
Leuconostoc spp.	*Bifidobacterium* spp.

Table 2 Microbiological and biochemical attributes of typical mesophilic lactic acid bacteria used in fermented milks

Characteristic	Lactococcus lactis *subsp.* lactis	Lactococcus lactis *subsp.* cremoris	Lactococcus lactis *subsp.* lactis *biovar* diacetylactis	Leuconostoc mesenteroides *subsp.* cremoris	Leuconostoc mesenteroides *subsp.* dextranicum
Cell shape and configuration	Cocci, pairs, short chains	Cocci, pairs, short/long chains	Cocci, pairs, short chains	Cocci, pairs, short/long chains	Cocci, pairs, chains
Catalase reaction					
Growth temperature (°C)					
Optimum	28–31	22	28	20–25	20–25
Minimum	8–10	8–10	8–10	4–10	4–10
Maximum	40	37–39	40	37	37
Incubation temperature (°C)	21–30	22–30	22–28	22	22
Heat tolerance (60 °C for 30 min)	±	±	±		
Lactic acid isomers	L(+)	L(+)	L(+)	D(−)	D(−)
Lactic acid produced in milk (%)	0.5–0.7	0.5–0.7	0.5–0.7	0.1–0.2	0.1–0.2
Acetic acid production (%)				0.2–0.4	0.2–0.4
Gas (CO_2) production			+	±	±
Proteolytic activity	+	+	+	±	±
Lipolytic activity	±	±	±	±	±
Citrate fermentation			+	+	+
Flavor/aroma compounds	+	+	+++	+++	+++
Mucopolysaccharide production	±	±	±	No dextran from sucrose	Dextran from sucrose
Hydrogen peroxide production	+	+	+	±	±
Alcohol production	±	±	±	±	±
Salt tolerance (% max.)	4–6.5	4.0	4–6.5	6.5	6.5

Adapted from Heller KJ (2001) Probiotic bacteria in fermented foods: Product characteristics and starter organisms. *American Journal of Clinical Nutrition* 73(supplement): 374S–379S; Pettersson HE (1988) Starters for fermented milks. 2. Mesophilic starter cultures. *International Dairy Federation Bulletin* 20–28.

starters consisting of undefined strains, and the defined starters. Examples of the defined thermophilic starter culture systems are (1) *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, where growth and acid production are enhanced owing to the protocooperative relationship between these two species, (2) *Lb. acidophilus* used for the production of acidophilus milk, and (3) *Lb. paracasei* subsp. *paracasei* for the production of yakult (*see* **Lactic Acid Bacteria**: *Lactobacillus* spp.: *Lactobacillus acidophilus*; *Lactobacillus* spp.: *Lactobacillus casei* Group; *Lactobacillus* spp.: *Lactobacillus delbrueckii* Group; *Streptococcus thermophilus*).

Manufacture of Starter Cultures

The traditional method for the production of bulk starter commences with stock culture, liquid, freeze-dried, or frozen at −196 °C, which is inoculated and prepared in a small volume (approximately 100 ml of

Table 3 Characteristics of thermophilic starters

Characteristic	Streptococcus thermophilus	Lactobacillus delbrueckii *subsp*. bulgaricus	Lactobacillus acidophilus	Lactobacillus casei *subsp*. casei	Bifidobacterium bifidum
Cell shape and configuration	Spherical to ovoid, pairs to long chains	Rods with round ends, single, short chains, metachromatic granules	Rods with round ends, single, pairs, short chains, no metachromatic granules	Rods with square ends, short/ long chains	Curved rods with bifurcated ends (Y shaped); can also be multibranched
Catalase reaction					
Growth temperature (°C)					
Optimum	40–45	40–45	37	37	37
Minimum	20	22	20–22	15–20	22
Maximum	50	52	45–48	40–45	48
Incubation temperature (°C)	40–45	42	37	37	37
Heat tolerance (60 °C for 30 min)	++	+			
Lactic acid isomers	L(+)	L(−)	DL	L(+)	L(+)
Lactic acid produced in milk (%)	0.6–0.8	1.7–1.8	0.3–2.0	1.2–1.5	0.1–1.4
Acetic acid production (%)	Trace	Trace	+	+	+++
Gas (CO_2) production					±
Proteolytic activity	±	+	±	±	+
Lipolytic activity	±	±	±	±	±
Citrate fermentation					
Flavor/aroma compounds	++	++	+	±	++
Mucopolysaccharide production	±	++	−	±	++
Hydrogen peroxide production	±	+	+	+	+
Alcohol production		Trace	Trace	Trace	Trace
Salt tolerance (% max.)	2.0	2.0	6.5	2.0	2.0

Adapted from Heller KJ (2001) Probiotic bacteria in fermented foods: Product characteristics and starter organisms. *American Journal of Clinical Nutrition* 73(supplement): 374S–379S; Dellaglio F (1988) Starters for fermented milks. 3. Thermophilic starters. *International Dairy Federation Bulletin* 227: 27–34.

starter growth medium) as 'mother culture', which in turn is subcultured daily into three or more bottles, with the best bottle selected for bulk starter production. This is followed by inoculation, using 1% inoculum, into larger volumes of the growth medium, that is, into 'feeder' or intermediate culture, and finally into the bulk starter unit. This method is still widely used, even though the propagation procedure is time consuming, requiring skilled operators, and may expose the culture to bacteriophage infection, which is one of the major hazards in the industry.

Milk is the usual growth medium for bulk starters, but other media may also be used. These may contain nonfat milk powder, phosphate salts, sodium citrate, dextrose, dextrin, pancreatin, dried autolyzed yeast, lactose, and sucrose.

It is essential that starter cultures are preserved so that stock cultures are always available in case of starter failure. In addition, successive subculturing can lead to the emergence of mutant strains, which may alter the overall behavior and general characteristics of the starter. Too many transfers may result in the loss of certain functional plasmids such as Lac^- or Prt^-.

Starter cultures for fermented milk manufacture are also available in freeze-dried or frozen concentrated form, and either as direct-vat-set (DVS) type or as cultures for bulk starter production. The popularity of DVS type cultures is increasing. Application of DVS cultures eliminates the risk of phage contamination during starter preparation in the plant and ensures appropriate strain balance.

In the mid-1960s, vacuum-dried cultures were replaced by frozen concentrated cultures. Frozen concentrated cultures contain 10^{10}–10^{11} cfu g^{-1}, a sufficient concentration to allow 70 ml to inoculate 1000 l medium for the bulk culture preparation. Preparation of frozen concentrated cultures involves growing cultures under optimal pH conditions, harvesting cells by centrifugation or ultrafiltration, standardizing the cell suspension to a specified activity, adding a cryoprotectant (glycerol, monosodium glutamate, sucrose, lactose, or skim milk), packaging, rapid freezing in liquid nitrogen (−196 °C) or in a dry ice–ethanol mixture, and storing in a deep freeze. The pH of the cell concentrate should be 6.6 for lactococci and 5.4–5.8 for lactobacilli.

Frozen concentrated starters can be used daily or as stand-by or emergency starter stock. Bacteria in frozen starter cultures are immediately active on thawing. They do not go through the lag phase when added to the vat.

In 1982, a French culture company, Eurozyme, introduced a new type of DVS culture, concentrated and freeze-dried. It is available in aluminum pouches under nitrogen. Each package may contain a single strain or a blend of defined strains, which are individually propagated, concentrated, freeze-dried (to less than 3% moisture), and then blended to attain specified activity. Each pouch has a starter population of approximately 10^{11} cells g^{-1}, does not require deep freeze storage temperatures, and can be stored at 4 °C for 1 year without loss of activity.

The major disadvantage of using freeze-dried concentrate cultures is the longer lag phase in the vat, adding an additional 30–60 min to the time required to ferment milk.

It has been demonstrated that most lactic acid bacteria can be successfully preserved by freeze-drying, with the exception of *Lb. delbrueckii* subsp. *bulgaricus* and *Lb. helveticus*. The optimum rehydration temperature for both mesophilic and thermophilic lactic acid bacteria is 20 °C.

Inhibition of Starter Cultures

A number of factors may adversely affect the activity of starters, leading to poor quality of fermented milks and financial losses to the manufacturer. When lactic acid is not produced by a starter culture at the desired rate, the culture is called 'slow'. The slowness can be due to either the genetic makeup of the strains or extrinsic factors. The latter include, among others, (1) bacteriophage, (2) residues of antibiotics and sanitizing agents, (3) inhibitory compounds naturally found in milk, (4) variations in milk composition due to mastitis or seasonal factors, and (5) metabolites of spoilage bacteria. A lower rate of acid production can also be caused by irregular culture transfers, by fluctuations in incubation temperature, and by overacidification.

Viability of a starter culture can be determined by the simple direct microscopic count on a methylene blue-stained slide. This method can also be used to assess the physiological state of the bacterial cells. The shape of old cells, cells exposed to excessive acidity and inhibitors, and those grown on solid media will be changed.

Various simple tests can be used to quantify the activity of starter cultures. The activity of yogurt starter bacteria can be measured by a decrease in pH or rise in titratable acidity of sterile 12% reconstituted skim milk incubated at 40 °C for 8 h. A pH of 4.2 and titratable acidity of 1.05% under the above conditions are expected.

Inhibiting Factors

Bacteriophages in the dairy plant come from lysogenic bacteria in the starter culture and may also originate on farms. It is not possible to eliminate the entry of phage into the dairy plant because raw milk continuously enters the facility. However, growth of the phage population can be controlled by effective sanitation.

In general, thermophilic starters are not affected as much as mesophilic starters. In recent times, phages of *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* have been reported, with *S. thermophilus* being relatively more susceptible than *Lb. delbrueckii* subsp. *bulgaricus*. In yogurt production, fermentation is relatively fast – it may take only 3–4 h. It is unlikely that all components of the culture would be simultaneously attacked by phages. In case of a phage attack on one strain, acid production by the unaffected strain/s will continue, causing few or no problems in production. Measures employed to minimize the risk of phage attack include (1) the use of mixed and phage-unrelated cultures, (2) strict adherence to aseptic techniques, and (3) proper heat treatment of the starter growth medium (e.g., at 85 °C for 30 min).

Residues of antibiotics and sanitizers such as quaternary ammonium compounds, iodophors, hypochlorites, and hydrogen peroxide inhibit the growth of starter cultures. They may contaminate milk as a result of human error. Antibiotics are used in the treatment of mastitis, while detergents and disinfectants are used for cleaning and sanitation purposes. In practice, it is necessary to ensure that the rinsing cycle is long enough to wash down these chemicals from the bulk starter tank. Starters differ in their susceptibility to antibiotics and sanitizers.

Natural inhibitors present in raw milk, namely, lactenins, the lactoperoxidase/hydrogen peroxide/thiocyanate system (LPS), agglutinins, and lysozyme, are generally destroyed by proper heat treatment prior to manufacture. However, mesophilic starter cultures are inhibited by the LPS system if the heat treatment of milk is milder, for example, after high-temperature short-time (HTST) pasteurization.

Starter growth and acid production are slower in abnormal milk, for example, in milk from mastitic cows or in milk with high hydrolytic rancidity. Seasonal variations in milk composition resulting in lower micronutrients (trace elements, nonprotein nitrogenous compounds) may also affect starter performance.

Progressive inhibition of acid production and a decline in the rate of acid production by the culture have been observed in yogurts produced with the addition of sucrose, which raises osmotic pressure in the system. The acid-producing ability of yogurt culture is fairly normal in mixes containing 4–7% sucrose. Commercial strains that are relatively osmotolerant may allow higher usage levels without delays in acid production during yogurt manufacture (*see* **Bacteriophage**: Biological Aspects; Technological Importance in the Diary Industry).

Exopolysaccharide Formation by Bacteria

In the early 1950s, the first mucoid variants of bifidobacteria were reported, followed by the characterization of 'bifidan', an exocellular polysaccharide (EPS) from a mucoid nonencapsulated strain of *Bifidobacterium bifidum*.

Thermophilic starters of yogurt, both *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*, can produce as much as 0.2% (w/w) of mucopolysaccharides after 10 days of fermentation. Likewise, the mesophilic lactic starter *Lc. lactis* subsp. *cremoris* is able to produce a capsular polysaccharide in the Swedish 'långfil', containing rhamnose, glucose, galactose, and glycerol.

Most of the polysaccharides produced in yogurt contain glucose and galactose along with minor quantities of fructose, mannose, arabinose, rhamnose, xylose, and *N*-acetylgalactosamine, individually or in combination. The polysaccharides form a network of filaments visible under scanning electron microscope. The bacterial cells are partly covered by polysaccharide and the filaments link the cells and milk proteins. The texture of yogurt results from a complex interaction between milk proteins, acid, and EPS produced, which may influence important physical properties such as firmness, smoothness, and viscosity, and gel stability or susceptibility to syneresis.

Ropy strains of yogurt starters are commercially available, and are particularly suitable for drinking yogurt and stirred yogurt production, contributing to smooth texture, higher viscosity, lower syneresis, and better tolerance of mechanical handling. The capsule also slows diffusion of lactic acid away from the cell, causing the cells to stop acid production sooner, which helps prevent overacidification of fermented milk. It is conceivable that some of the exopolysaccharides play a physiological role in the human digestive system because of their chemical structure resembling the fiber of grains and vegetables.

Bacteria that are known to produce EPS include *S. thermophilus, Lb. kefiranofaciens, Lb. helveticus, Lb. sake, Lb. delbrueckii* subsp. *bulgaricus, Lc. lactis* subsp. *cremoris, B. longum*, and *B. infantis*. Recent studies consider EPSs as having probiotic properties since they may contribute to human health either as a nondigestible food fraction or because of their claimed antitumoral activity. The industrial value of the EPS depends on their ability to modify the rheological properties of aqueous solutions. They have been traditionally used in the food industry as thickening, gelling, or stabilizing agents.

A number of important functions have been ascribed to EPS:

1. Promotion of adhesion to oral surfaces and biofilm formation
2. Improvement of viability after adhesion
3. Promotion of resistance to nonspecific host immunity

EPSs play an important role in the rheology, texture, and mouthfeel of fermented milks, and are found in yogurt, kefir, viili, and some other products.

Concluding Remarks

Starters are carefully selected microorganisms that are added to milk to initiate and carry out the desired fermentation. Precaution should be taken in starter culture preparation, especially while 'mother culture' is prepared in a traditional way, to hinder bacteriophage infection by effective aseptic and sanitation practices, as well as thorough attention to detail in all manufacturing steps.

See also: **Bacteria, Beneficial**: *Bifidobacterium* spp.: Applications in Fermented Milks. **Bacteriophage**: Biological Aspects; Technological Importance in the Diary Industry. **Biofilm Formation**. **Fermented Milks**: Nordic Fermented Milks; Types and Standards of Identity. **Lactic Acid Bacteria**: *Lactobacillus* spp.: *Lactobacillus acidophilus*; *Lactobacillus* spp.: *Lactobacillus casei* Group; *Lactobacillus* spp.: *Lactobacillus delbrueckii* Group; *Lactococcus lactis*; *Leuconostoc* spp.; *Streptococcus thermophilus*.

Further Reading

Charteris WP, Kelly PM, Morelli L, and Collins KJ (1998) Ingredient selection criteria for probiotic microorganisms in functional dairy foods. *International Journal of Dairy Technology* 51(4): 123–136.

Cogan TM and Accolas J-P (eds.) (1996) *Dairy Starter Cultures*. New York: Wiley-VCH.

Dellaglio F (1988) Starters for fermented milks. 3. Thermophilic starters. *International Dairy Federation Bulletin* 227: 27–34.

Frank JF and Hassan NA (1998) Starter cultures and their use. In: Marth EH and Steele JL (eds.) *Applied Dairy Microbiology*, pp. 131–172. New York: Marcel Dekker.

Heller KJ (2001) Probiotic bacteria in fermented foods: Product characteristics and starter organisms. *American Journal of Clinical Nutrition* 73(supplement): 374S–379S.

ISO 27205:2010 (IDF 149:2010) Fermented Milk Products–Bacterial Starter Cultures–Standard of Identity, pp.1–4.

Marshall VM (1993) Starter cultures for milk fermentation and their characteristics. *Journal of the Society of Dairy Technology* 46(2): 49–56.

Martine B, Roya D, and Vuillemard J-C (2000) Biochemical characteristics of fermented milk produced by mixed-cultures of lactic starters and bifidobacteria. *Le Lait* 80: 465–478.

Pettersson HE (1988) Starters for fermented milks. 2. Mesophilic starter cultures. *International Dairy Federation Bulletin* 20–28.

Tamime AY (1990) Microbiology of 'starter cultures'. In: Robinson RK (ed.) *Dairy Microbiology, Vol. 2: The Microbiology of Milk Products*, 2nd edn., pp. 131–201. London: Elsevier Applied Science.

Tamime AY, Skriver A, and Nilsson LE (2006) Starter cultures. In: Tamime AY (ed.) *Fermented Milk*, pp. 11–25. Blackwell Publishing Ltd.

Torino MI, Sesma F, and Font De Valdez G (2000) Semi-defined media for the exopolysaccharide (EPS) production by *Lactobacillus helveticus* ATCC 15807 and evaluation of the components interfering with the EPS quantification. *Milchwissenschaft* 55(6): 314–316.